ANNUAL REPORT

LABORATORY OF PHYSICAL BIOLOGY

NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

OD annual reports.

OCTOBER 1, 1987 THRU SEPTEMBER 30, 1988

Muscle Contraction

This year several new approaches were inaugurated for studying muscle fiber behavior under conditions where specific structural modifications were effected. For example, the influence of sulfhydryl reacting alkylating agents on actomyosin crossbridge strength was examined. Another tactic was to use several different techniques to change the content of the recently discovered megadalton proteins, titin and nebulin, in muscle fibers. In previous years, irradiation was used to degrade both titin and nebulin; this FY low concentrations of trypsin were used to selectively degrade titin and the physiological and structural properties of such fibers were studied. In addition further progress was made in the use of image processing to overcome problems associated with cryoelectronmicroscopy of muscle fibers (large scale distortion in frozen sections and the use of underfocussed imaging where specimen density is not conveyed in a linear manner). Comparison of X-ray diffraction patterns calculated from restored images with those obtained experimentally were found to give good agreement. This inspires confidence in the use of cryo techniques to study mass distribution in muscle in various physiologically interesting states.

The behavior of ATP-crossbridges.

The key element in understanding the ability of muscle to shorten and make force is understanding the interaction of the muscle myosin crossbridges with the actin filament. This laboratory first demonstrated and characterized the behavior of the muscle crossbridge state seen when ATP binds to the myosin nucleotide binding site. Initially, studies along these lines were restricted to measuring the rapid resting stiffness of muscle fibers bathed in low ionic strength solution, where, because of increased strength of binding, the experiments were most simple. In FY '88, several important bits of information were added to this. First, it was shown that, even at physiological ionic strength, rapid resting stiffness is a good measure of the behavior of ATP crossbridges. Second, and even more importantly, it was shown that the behavior of ATP crossbridges in fibers correlates almost perfectly with the behavior of actin and myosin subfragment-1 with ATP in solution. Thus not only was the behavior of an important state in the crossbridge cycle elucidated, but progress was made in understanding how one can predict intact muscle crossbridge behavior simply in terms of the solution biochemistry of the relevant proteins. (Schoenberg).

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The influence of crossbridge density on muscle behavior.

Several of the properties of muscle crossbridge behavior may depend critically on the interaction between nearest neighboring crossbridge heads. Thus a means was sought for decreasing the density of crossbridge heads while maintaining a functional muscle. One promising way of doing this seemed to be to treat the fiber with the bifunctional sulfhydryl reacting alkylating agent, pPDM. This agent is known to crosslink the SH_1 and SH_2 sulfhydryls of myosin and greatly weaken the ability of myosin to interact with actin. However, this treatment was found to have a very serious side effect in fibers; it caused significant increases in the control values of resting tension. Fortunately, the equivalent monofunctional alkylating agent, phenylmaleimide, was discovered to decrease crossbridge affinity without affecting resting tension. This finding not only refutes the previous idea that actual crosslinking of SH_1 and SH_2 is necessary for reducing crossbridge affinity, it possibly opens the way for studying muscle behavior under conditions where the density of crossbridges capable of interacting with actin is greatly reduced (Schoenberg, Barnett).

Regulation of smooth muscle.

The prevailing view is that smooth muscle is activated when Ca^{2+}-calmodulin sensitive myosin light chain kinase (MLCK) phosphorylates serine-19 of the 20 kD mysoin light chains. A mystery is why, during the later stages of a contraction, force is maintained even though light chain phosphorylation returns to near baseline values. This phenomenon has been thought possibly to be related to the action of protein kinase C, which, *in vitro*, phosphorylates the myosin light chains at serine-1. To test this hypothesis, the phorbol ester stimulator of protein kinase C, phorbol-12,13 dibutyrate (PDB), was used to induce a contraction in bovine tracheal smooth muscle and the quantity and site of mysoin light chain phosphorylation was followed. Stimulation of contraction with PDB results in but slight elevations of myosin light chain phosphorylation, and the major site of phosphorylation is the site phosphorylated by MLCK rather than protein kinase C. This suggests that the activation of smooth muscle by PDB is due to partial activation of MLCK, possibly related to the ability of PDB to cause Ca^{2+} mobilization. The activation does not seem to be related to direct phosphorylation of the myosin light chains by protein kinase C, thus suggesting that other mechanisms must be responsible for maintaining high tension in the face decreased light chain phosphorylation late in contraction (Schoenberg, Corson).

Reconstruction of the equatorial diffraction pattern.

Efforts in improving our understanding of the two dimensional density maps of muscle cells reconstructed from experimental X-ray data have continued. To reconstruct a molecular structure faithfully, a large number of reflections is needed. However, since the packing of myofilaments in muscle is not exactly crystalline, only a limited

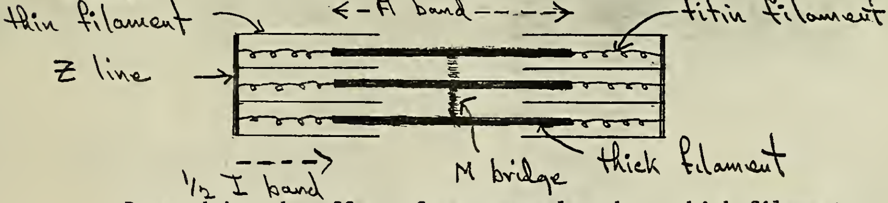
number of reflections has been observed (a maximum of 12 on the equator so far). Model calculations show that some features in the reconstructed density maps based on 5 or 7 reflections could be caused by effects of series termination, i.e. terminating the Fourier synthesis at a limited number of terms. However, through model calculations, it is possible to estimate, albeit approximately, the underlying density distributions derived from the experimental data without spurious features. Therefore, interpretations of the density maps at limited resolution could be deconvoluted from the effects of series termination. (Yu).

Cardiac muscle.

Equatorial diffraction patterns were obtained from intact and skinned cardiac (trabeculae) muscle preparations to determine the interfilament spacings at various degrees of stretch. Lattice spacing is strongly dependent on stretch, even in the skinned muscle preparation, where the outer membrane has been disrupted. The spacing is approximately in inverse proportion to the amount of stretch beyond the rest length. If confirmed by further experiments, the findings may have direct relevance to understanding the mechanisms underlying the steep length dependence of mechanical output in cardiac muscle at the molecular level (Yu, Martyn).

Physiological role of titin.

Muscle fibers generate force by lateral interaction of two sets of inelastic filaments: the thick, myosin-containing filaments and the thin, actin-containing filaments. As diagrammed below, the thin filaments originate at the Z line and extend toward the middle of the sarcomere. In relaxed fibers the thick filaments are located in the center of the sarcomere, which is the optimal position for force generation, but they tend to move away from the center when fibers are activated. If some mechanism were not available to recenter the thick filaments, successive contractions would develop progressively smaller forces.



By studying the effect of sarcomere length on thick filament movement in activated fibers, evidence was found that thick filaments are centered in the sarcomere by means of elastic titin filament linking the thick filaments to the Z line. In particular, the central position of the thick filaments in activated fibers was unstable in short sarcomeres (sarcomere length less than 2.8 mμ) but was stabilized when the sarcomere length was raised above 2.8 mμ. This difference of thick filament behavior in the two ranges of sarcomere length is consistent with the filament arrangement in the diagram and

is caused by the effect of sarcomere length on the stiffness of the titin filaments. At the short sarcomere length the titin filaments are not stiff enough to prevent the thick filaments from moving away from the center, while at longer sarcomere lengths the titin stiffness increases and can prevent thick filament motion.

The velocity of thick filament movement at short sarcomere lengths is very slow on the physiological time scale. The parameters affecting the kinetics of thick filament movement were studied by modelling. The slowness of the movement was found to be caused by the strong resistance of actomyosin crossbridges on one-half of the filament to being "pulled"; the force transmitted by strained titin filaments to the thick filaments did not significantly retard the motion. Therefore, the primary function of the titin filaments _in vivo_ is to keep the thick filaments centered during passive stretch and to prevent sarcomere asymmetry from accumulating over several contractions by recentering the thick filaments each time the muscle is relaxed (Horowits, Podolsky).

Effects of titin degradation.

Irradiation of muscle fibers degrades the megadalton proteins, titin and nebulin, without affecting the smaller structural proteins (myosin, actin, tropomyosin, troponin), and leads to a drop in both resting tension and the force developed by calcium-activation. The equatorial X-ray diffraction patterns are also affected by irradiation: the intensities of the 10 and the 11 reflections are reduced, relative to the background, when fibers are irradiated in both the relaxed and the rigor (ATP-free) states. Preliminary electronmicroscopy of such fibers indicates that irradiation reduces the diameter of the thick filaments. These results are consistent with the idea that titin (a) in the A band is attached to the outer surface of the thick filament, and (b) passes through the I band and joins the thick filament to the Z line (Tanaka, Barbosa, Zhao, Podolsky).

Titin can be degraded without affecting nebulin by treating fibers with a low concentration of trypsin. In this case the equatorial X-ray diffraction pattern of fibers treated in the relaxed state is degrades, but the diffraction pattern of fibers treated in the rigor state is unchanged. This result suggests that trypsin degraded titin differently than irradiation or that nebulin affects the strength of the rigor crossbridges (Tanaka, Podolsky).

Thick filament superlattice.

The central part of the thick filaments in rabbit skeletal muscle is triangular in cross section, and these triangles are arranged in a superlattice obeying the "no-three-alike" rule. The superlattice requires the presence of M bridges in the middle of the sarcomere because it is degraded when the M bridges are removed by low ionic strength, high pH solutions (Barbosa, Zhao).

Two dimensional X-ray detector.

A new imaging X-ray detector, formed by coupling a gadolinium oxysulfide phosphor to the input of an optical imaging photon detector, was characterized. The device is small, light, easy to use, and features a direct digital readout. It has been used to record good two-dimensional diffraction patterns from striated rabbit muscle in less that one-tenth the time needed under similar conditions using film (Collett, Podolsky).

High Resolution Electron Microscopy

Intermediate filaments

Intermediate filaments (IF) are one of the three major components of the cytoskeletons of virtually all eukaryotic cells, the others being microfilaments (actin) and microtubules (tubulin). IF proteins are known to form two-chain molecules that are ~48nm in length, with the two polypeptide chains joined via an α-helical coiled-coil rope formed by their respective "rod" domains. Over the past year, information has been gathered on how IF molecules associate into structure at the next higher level. Vimentin protein was purified from BHK cells and reduced to soluble form by low ionic strength and temperature, then prepared for electron microscopy. The purpose was to see if the particles thus visualized would fall into particular (e.g. quantized) length classes. The main class of particles were, as considered likely from previous work, 40-50nm long. However, some particles were folded back (thicker 20-25nm particles), and others were L-shaped with a joint in the middle. These data provide what is perhaps the best evidence to date that IF molecules fall into two subdomains of approximately equal length, this division having been inferred on the basis of an interruption in the progression of heptads near the middle of the rod domain sequence.

These proteins were also examined in the STEM after freeze-drying. Such data have the advantage of allowing measurements of mass as well as of length, so that direct estimates of the number of molecules involved in each complex can be made. However, with such small and wispy particles, the signal-to-noise ratio is marginal, and their analysis has required the development of a special computer program. The preliminary results are morphologically consistent with the shadowing data (cf. above) but also show that most of the particles are simple two-chain molecules, and not tetramers or higher aggregates (Steven, Steinert, Trus).

Cryomicroscopy of vertebrate skeletal muscle.

For the purpose of determining net interactions between actin and myosin filaments in muscle cells, perhaps the single most informative view of the myofilament lattice is its averaged axial projection. Frozen-hydrated transverse thin sections were studied with the goal of obtaining axial projections that are not subject to limitation of conventional thin sectioning (suspect preservation of native structure) or of equatorial X-ray diffraction analysis (lack of

experimental phases). In principle, good preservation of native structure may be achieved with fast freezing, followed by low-dose electron imaging of unstained vitrified cryo-sections. In practice, however, cryo-sections undergo large-scale distortions, including irreversible compression; furthermore, phase contrast imaging results in a non-linear relationship between the projected density of the specimen and the optical density of the micrograph.

To overcome these limitations methods of image restoration and generalized correlation averaging were devised and applied to cryo-sections of rabbit psoas fibers in both the relaxed and rigor states. At ~10nm resolution, myosin filaments appear less prominent relative to actin filaments, compared to what is seen in conventional thin sections. This may indicate that a significant fraction of their crossbridges averages out in projection so as to contribute only to the baseline of projected density. Entering rigor encurs a loss of density from an annulus around the actin filament. This redistribution of mass represents attachment of the "visible" fraction of crossbridges. Myosin filaments in the "non-overlap" zone broaden visibly on entering rigor, suggesting that these crossbridges move outwards even without actin in their immediate proximity. The intensities of the two strongest reflections in the equatorial X-ray diffraction pattern, I_{10} and I_{11}, were calculated from the mass distribution and found to be close to experimentally measured values. This result makes it likely that the cryo and image processing techniques used have are free of significant artifact (Steven, Trus, Unser, Barbosa, Yu, Podolsky).

Outer membrane proteins of B. pertussis.

This project involves investigation of a crystalline two-dimensional lattice that was observed earlier as being present on the surfaces of two non-pathogenic strains of B. pertussis. High resolution negative staining and computational filtering techniques were applied to cell envelopes of these bacteria. It was found, rather unexpectedly, that these structures are not S-layer ("surface layer") arrays of extrinsic high-M_r glycoproteins such as are present on many Gram-negative and Gram-positive bacteria. Rather, the B. pertussis arrays are natural crystals of a 40K intrinsic membrane protein that has the properties of an anion-selective porin. Porins are an extensive and much-studied family of channel-forming proteins that render outer membranes permeable to solutes of up to ~650 Da. Future plans include a three-dimensional reconstruction of this structure in negative stain, frozen-hydrated electron microscopy, and attempts to obtain highly ordered crystals in 2 dimensions (for high resolution electron microscopy studies) and 3 dimensions (for X-ray crystallography) (Kessel, Trus, Steven, Brennan).

Cell Membrane

Mechanism of assembly and disassembly.

Much of the recent experimental work is related to confirming previous observations that the assembly of membrane bilayer occurs at a critical temperature T*, the growth temperature of the cell. Two aspects of this property were examined: How general is the observation that T* is the growth temperature of the cell? Since T* is a critical point, and therefore represents the condition of optimal membrane stability, how will violations of the stability conditions be manifested in membrane behavior?

T* is obtained by measuring the surface pressures of aqueous dispersions prepared from the total lipid extracts of various cell membranes as a function of temperature. The agreement between T* and growth temperature has previously been demonstrated for several bacterial and red blood cells. It has now also been demonstrated with total lipid extracts of rat cerebellum, cortex and brain stem, where T* is 36 ± 1 °C, and of squid fin and optic nerve where T* is 15 ± 1 °C. For squid, spawning temperatures are approximately 16 °C. Thus, the concept that T* is equal to the growth temperature of cellular systems is applicable to a broad range of cellular species, and over a wide range of growth temperatures (15-60 °C) (Gershfeld).

The concept that T* is the temperature of optimal stability of cell membranes was tested by measuring hemolysis rates for human red blood cells as a function of temperature. As predicted, even small elevations above normal body temperature resulted in hemolysis. Under conditions of pyrexia the rates of hemolysis are extremely low and increase with temperature. Cooling the red blood cells below normal body temperatures resulted in stable conditions and no hemolysis. This apparent violation of the critical bilayer state theory, was resolved by stability studies with artificial bilayers; when heated above T*, the unilamellar state breaks down, but cooling below T* resulted in supercooling of the bilayer. Thus, it is believed that red blood cells do not hemolyze when cooled below body temperature because the membrane bilayer supercools and remains as a stable structure (Gershfeld and Murayama).

Platelet Aggregation

The main question in this work has to do with the influence of pressure on the blood coagulation system, including both hemostasis and thrombosis. The findings emphasize the important role of the water molecule in these biochemical reactions. Platelet aggregation takes place because oppositely charged amino acids are juxtaposed at the binding site (Arg-Gly-Asp-Ser). Normally, charged residues are surrounded by water (small dipolar molecules). These organized water molecules are arranged compactly around the charged residues. When this shield of water is made thinner by increased escaping tendency the oppositely charged residues interact and the volume of the solution is increased by water molecules which are randomized into the bulk phase. Platelet aggregation is inhibited by increasing the concentration of neutral salts, because counterions surround the

charged residues, thus weakening the charge effect. For the same reason fibrin polymers dissolve in salt solution (1 M NaBr). Molecules having a large electric dipole moment inhibit decompression-inducible platelet aggregation (DIPA) by essentially the same mechanism as if the ionic strength were increased. This was confirmed experimentally. Piracetam (N-acetamide of GABA) has a large electric dipole moment which can block both fibrin polymerization and DIPA at a low concentration (1 mM); the rate of fibrin polymerization is decreased 50% by 9 mM piracetam (Murayama).

Radiation Inactivation Analysis

Binding sites for higher density lipoproteins.

Studies in human fibroblasts and liver revealed that the radiation-sensitive binding site was quite small (16 kilodaltons (kDa) in fibroblasts, <8 kDa in liver). Although these binding sites had all of the pharmacoloical properties of putative receptor sites, the target size was unprecedently small, suggesting that such binding does not occur to a conventional protein receptor. Similar radiation effects had been previously reported for cholera toxin binding in thyroid membranes which has now been shown to bind to gangliosides (Kempner).

Binding of GTP by p21 ras protein.

The p21 ras proteins currently are of great interest because of their association with oncogenes. It has been established that p21 avidly bind GTP, and this was used as a measurable function for target analysis. Radiation inactivation revealed a size approximately 3 times larger than the known monomer structure. A similar target size was found for the disappearance of the monomer protein on electrophoretograms, suggesting that 2-4 polymers were destroyed by one radition event. This result obscures that interpretation of the minimum structure for GTP binding. However, the two alternative models (one GTP molecule bound per 3 p21 proteins or one-for-one) could be resolved by examination of the highest specific binding activity which was obtainable in purified p21 proteins. GTP bound per p21 was always found to be less than 0.4, suggesting that an oligomeric structure is the true binding site (Kempner).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27000-26-LPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Mechanism of Muscular Contraction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard J. Podolsky	Chief	LPB/NIAMS
Others:	Leepo C. Yu	Research Physicist	LPB/NIAMS
	Robert Horowits	Staff Fellow	LPB/NIAMS
	Maria Barbosa	NRSA	LPB/NIAMS
	Hidehiro Tanaka	Visiting Scientist	LPB/NIAMS
	Fu Zhao	Visiting Scientist	LPB/NIAMS

COOPERATING UNITS *(if any)* Dr. Alasdair Steven, Section on Structural Biology, LPB; Dr. Ellis Kempner, Section on Macromolecular Biophysics, LPB; Dr. Marinos Dalakas, NINCDS.

LAB/BRANCH Laboratory of Physical Biology

SECTION Section on Muscle Biophysics

INSTITUTE AND LOCATION NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 7.5 PROFESSIONAL: 5.5 OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Striated muscle cells require a high degree of axial order to function properly. This FY the properties of the elastic titin filaments which link the ends of the thick, myosin-containing filaments to the nearest Z line were characterized. The central position of the thick filaments in the sarcomere, which is optimal for force generation, is intrinsically unstable. It was found that thick filament movement away from the center is slow on the physiological time scale. This slowness is due primarily to properties of the actomyosin crossbridges. The main role of the titin filaments *in vivo* is to keep the thick filaments centered during passive stretch of the muscle fiber and by recentering the thick filaments each time the muscle fiber is relaxed to prevent sarcomere asymmetry from adding up over several contractions.

The influence of degradation of titin alone (by treatment with low concentrations of trypsin) or of titin plus nebulin (produced by high energy irradiation) on muscle structure was studied by X-ray diffraction. Both procedures caused the intensities of the equatorial reflections to decrease in relaxed fibers. Enzymatic treatment of rigor fibers did not affect the X-ray pattern, although irradiation caused the X-ray pattern to weaken. Electron microscopy suggests that both treatments bring about a reduction in thick filament diameter, which indicates that titin is attached to the outer surface of the thick filaments.

Cryomicroscopy and image processing were used to obtain the axially projected mass distribution in relaxed and rigor rabbit muscle, and the equatorial X-ray diffraction pattern was calculated. The calculated intensities of the 10 and 11 reflections were close to the directly measured results, which makes it likely that the cryo and image processing techniques are free of artifact.

A new imaging X-ray detector was characterized. It has been used to record good diffraction patterns from striated rabbit muscle in less than one tenth the time needed under similar conditions using film.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 27001-14 LPB

PERIOD COVERED
October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Contractility of Skeletal and Smooth Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. Schoenberg, Medical Officer, LPB, NIAMS

Others: V. A. Barnett, Staff Fellow, LPB, NIAMS

COOPERATING UNITS *(if any)*
Laboratory of Molecular Cardiology, NHLBI (R. S. Adelstein, J. R. Sellers, M. A. Corson)

LAB/BRANCH
Laboratory of Physical Biology

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2.0	2.0	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Key in understanding the ability of muscles to shorten and make force is understanding the interaction of myosin crossbridges with the actin filament. We have been involved in characterizing the behavior of myosin crossbridges when they have ATP bound. During FY '88 we substantiated that at ionic strengths ranging from very low values up to physiological values, measuring the muscle fiber's rapid resting stiffness provides an accurate way of monitoring the interaction of these ATP crossbridges with actin. It was furthermore shown that the binding of the ATP crossbridge in fibers correlates virtually perfectly with the binding of myosin subfragment-1 to actin in solution. Since the solution work is done in the absence of the regulatory proteins troponin and tropomyosin, this suggests that the increased binding of ATP crossbridges at low ionic strength is a direct effect of ionic strength on the crossbridge rate constants, unrelated to any changes in troponin and tropomyosin structure which may also occur.

Also in FY '88, we looked for ways of studying the influence of crossbridge density on crossbridge behavior. Our plan was to modify just some of the crossbridges in a way that would render them incapable of binding to actin. We tried treating fibers with the bifunctional alkylating agent pPDM, which, in solution, greatly weakens actin binding affinity by crosslinking myosin's SH1 and SH2 sulfhydryls. In the fiber, however, this had the deleterious side effect of increasing the fiber's resting tension. We discovered that the monofunctional reagent phenylmaleimide could reduce crossbridge affinity without increasing resting tension. This showed that crosslinking of SH1 and SH2 is not necessary for reducing crossbridge affinity and may also have provided us with a tool for studying the influence of crossbridge density on crossbridge behavior.

Finally in FY '88, we demonstrated that the ability of smooth muscle to maintain force even after myosin light chain phosphorylation, the trigger for force generation, has returned to near baseline values, is not due to protein kinase C phosphorylating the myosin light chains at serine-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27002-10 LPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Macromolecular Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Alasdair C. Steven, Visiting Scientist - Section Chief	LPB, NIAMS
Adelia C. Bauer, Physiologist	LPB, NIAMS
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Frank P. Booy, Visiting Scientist	LPB, NIAMS

COOPERATING UNITS *(if any)*

Computer Systems Laboratory, Division Computer Research and Technology, NIH (B.L. Trus); Biology Department, Brookhaven Laboratory, (J. Hainfeld, J. Wall); Other collaborators as specified below.

LAB/BRANCH

Laboratory of Physical Biology

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☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structures and mechanisms of assembly of biological macromolecules and macromolecular complexes are studied, primarily by high resolution electron microscopy and computer image processing, but also incorporating other information such as amino-acid sequence analyses. Over the past year, we have made headway primarily in (1) analysis of frozen-hydrated transverse thin sections of relaxed and rigor skeletal muscle; (2) formulating a molecular model for the fibrous receptor-recognition protein of reovirus; and (3) characterizing the regular surface lattice found in the outer membranes of two strains of Bordetella pertussis.

(1) After restoring the cryo-section images for non-linear contrast transfer correcting for spatial disorders, and averaging to improve the signal-to-noise ratio, we have observed the distribution of mass within the projected unit cell of the myofilament lattice in both states. On entering rigor, mass is transferred from the periphery of the myosin filaments to surround the actin filaments. We also observe changes to affect myosin filaments in rigor even in the absence of actin.

(2) Sigma-1 protein fibers released from reovirions upon heating are homotetrameric molecules, 48nm long x 5-9nm wide. The distal part of the rod is made up of two coiled-coil helical ropes placed side-by-side. This segment is followed by a wider portion of about the same length as the first (23nm) but which is wider, contains visible substructure, and is rich in beta-sheet conformation. Finally, the carboxy termini form globular domains where the receptor-combining sites reside.

(3) Applying generalized image averaging techniques, we have found that the B. pertussis surface lattice is rectangular and observes p2 symmetry. Its unit cell contains two trimers of the bacterium's 40K anion-selective porin, in different orientations. That these porins are active in the crystalline state is implied by the normal growth rate of these strains that exhibit this structure.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27003-29 LPB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biophysical Studies of Metabolic Activity and Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ellis S. Kempner, Ph.D., Physicist and Chief, Section on Macromolecular Biophysics LPB NIAMS

COOPERATING UNITS (if any)
Drs. C.M. Mendel, A.S. Verkman, and S. Prusiner (University of California); Dr. S. Fleischer (Vanderbilt University).

LAB/BRANCH
Lab/Branch: Laboratory of Physical Biology

SECTION
Section: Section on Macromolecular Biophysics

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1) Target analysis of the inactivation of biological activity by ionizing radiation was applied to three major problems: a) the binding sites for high density lipoproteins in different tissues; the binding of GTP by p21 *ras* proteins; and c) the infectious agent for scrapie.
2) Major reviews of previous radiation studies of enzymes and of membrane-associated functions were completed.
3) Work was completed on a general theoretical description of the effects of ionizing radiation which are unique to macromolecules.

Objectives: 1) An understanding of the nature of active structures *in vivo* which are involved in biochemical processes, principally by means of the technique of inactivation by ionizing radiation.
2) Detailed knowledge of the molecular damage caused by ionizing radiation and of the mechanisms of the transfer of radiation energy throughout these structures.

Methods: 1) General biochemical techniques including enzyme reactions, fluorescence, and gel electrophoresis.
2) Ionizing radiation, usually high energy electrons from a linear accelerator, to expose samples under carefully controlled conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 27004-19 LPB

PERIOD COVERED
October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Dynamic Properties of Cell Membranes and Related Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Dr. Ralph J. Nossal PSL, DCRT and Dr. Stanley I. Rapoport LN, NIA

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  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principal focus of this project is to describe the molecular mechanisms that cells utilize for membrane assembly and disassembly. Previous reports have presented evidence showing that membrane lipid bilayer assembly is a critical phenomenon in which the growth temperature is a critical point. The critical temperature T* has now been demonstrated for the total lipid extracts of B. stearothermophilus, E. coli human red blood cells, rat brain, and the optic nerve of squid (Loligo). These studies span cell temperatures ranging from 15-60 C. From the critical bilayer assembly theory two conditions are predicted to cause membrane bilayer structure degeneration: a) when cell temperature is raised with membrane lipid composition kept constant, and b) when membrane lipid composition is modified at constant cell temperature. The first condition of membrane degeneration has now been demonstrated with studies of red blood cells under conditions of pyrexia. Hemolysis has been observed even when temperatures are elevated only 1 degree above 37 C; no hemolysis is detected at 37 C. The measured activation energy for hemolysis is consistent with theoretical calculations for membrane bilayer degeneration based on critical state theory. For testing the second condition for membrane degeneration (modification of membrane lipids) a comparison has been made of critical bilayer assembly for lipid extracts of tissue from Alzheimer's disease brains. Extracted lipids obtained from tissues with lesions characteristic of the disease do not assemble membrane bilayers at body temperature, while the lipids from tissue free of lesions assemble membrane bilayers at body temperature. From the perspective of the critical bilayer assembly theory these experiments indicate that a defect in the lipids obtained from Alzheimer lesions will cause membrane bilayer breakdown. The chemical nature of the lipid defect is currently under examination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27005-06 LPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aggregation of Human Platelets Induced by Decompression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Makio Murayama Research Chemist LPB, NIAMS

COOPERATING UNITS *(if any)*

Dr. K.K. Kumaroo, Biochemist, U.S. Naval Medical Research Institute, Bethesda, MD

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main thrust of the laboratory is focussed on the influence of pressure on the blood coagulation system, including both hemostasis and thrombosis. However, our findings emphasize the important role of the water molecule in these biochemical reactions. Platelet aggregation takes place because oppositely charged amino acids are juxtaposed at the binding site (Arg-Gly-Asp-Ser). Normally, charged residues are surrounded by water (small dipolar molecules). These organized water molecules are arranged compactly around the charged residues. When this shield of water is made thinner by increased escaping tendency the oppositely charged residues interact and the volume of the solution is increased by water molecules which are randomized into the bulk phase. Platelet aggregation is inhibited by increasing the concentration of neutral salts, because counterions surround the charged residues, thus weakening the charge effect. For the same reason fibrin polymers dissolve in salt solution (1 M NaBr). Molecules having a large electric dipole moment inhibit decompression- inducible platelet aggregation (DIPA) by essentially the same mechanism as if the ionic strength were increased. This was confirmed experimentally. Piracetam (N-acetamide of GABA) has a large electric dipole moment (6.7 Debye units) which can block both fibrin polymerization and DIPA at a low concentration (1 mM); the rate of fibrin polymerization is decreased 50% by 9 mM piracetam.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27012-04 LPB

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural and Mechanical Properties of Muscle Fibers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Leepo C. Yu, Research Physicist, Laboratory of Physical Biology, NIAMS

COOPERATING UNITS *(if any)*

Dr. Bernhard Brenner (University of Tubingen, FRG)
Dr. Don Martyn (University of Washington, Seattle)

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INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The present project investigates the structural aspect of interactions between myosin and actin (crossbridges) in muscle cells.

(i) We characterized the binding of myosin to actin, i.e. formation of crossbridges, in the presence of ATPgS, a slowly hydrolyzable ATP analogue. The binding characteristics indicate that the ATPgS-myosin binds to actin strongly and the binding is cooperative in the absence of calcium. Thus the ATPgS-crossbridge state resembles those states accompanying or following the release of phosphate in the actomyosin ATPase cycle. This ATP analogue binds to myosin at nearly full saturation level, resulting in a pure ATPgS-crossbridge state, which could considerably facilitate studies of the strong binding states.

(ii) Calculations showed that some of the features in the density maps reconstructed from five or seven equatorial reflections could be due to effects of insufficient terms used in the Fourier synthesis. However, through model calculations, it is possible to estimate, albeit approximately, the underlying density distributions derived from experimental data without the spurious features.

(iii) Equatorial X-ray diffraction studies on intact and skinned cardiac muscle have been initiated. Preliminary results indicate that the interfilament spacing is inversely proportional to the amount of stretch beyond rest length, even in the skinned preparation.

Annual Report of the Arthritis and Rheumatism Branch
National Institute of Arthritis and Musculoskeletal and Skin Diseases
October 1, 1987 through September 30, 1988

The Branch's professional activities include both clinical and basic laboratory investigations. The clinical research program is focused on therapeutic and investigations. The clinical research program is focused on therapeutic and investigational protocols of immune-mediated diseases, primarily systemic lupus erythematosus, polymyositis, and rheumatoid arthritis. The clinical program is directed by four Senior Staff physicians who serve as principal investigators of these studies. During the past year, these studies accounted for approximately 450 patient admissions as well as over 2000 follow-up outpatient visits. In addition, the Branch responded to some 100 requests for rheumatology consultations for patients involved in research studies of other NIH Institutes.

The Branch conducts a rheumatology training program for physicians who wish to pursue careers as independent clinical investigators. A total of seven individuals participate through the Medical Staff Fellow Program.

The laboratory investigations involve studies on animal models of lupus, arthritis and polymyositis as well as fundamental studies at the cellular and molecular level on various aspects of the immune and inflammatory response, and in the orthopedic research unit, on bone growth and development normally and after trauma.

I. Disease-related Studies

A. Rheumatoid Arthritis

1. Pathogenesis

a. Animal Models(48)*

Bacterial cell wall-induced arthritis is an experimental animal model of chronic proliferative and erosive synovitis that is induced by systemic administration of biodegradation resistant bacterial cell wall fragments in aqueous solution. The clinical, pathological, biochemical and immunological features of the model closely resemble rheumatoid arthritis. The studies conducted by Dr. Ronald Wilder's group have the following goals.

i. To define the properties of bacterial cell walls that facilitate the development of arthritis.

ii. To characterize the host genetic factors that regulate susceptibility to developing arthritis.

iii. To better define pathophysiologic processes by characterizing differences between high and low responder inbred rat strains.

iv. To define mechanisms leading to tissue injury and destruction and

v. To develop techniques to modulate the pathologic process.

During the past year the following major findings were made:

i. Transin, a gene expressed intensely in transformed fibroblasts is expressed intensely in the synovial tissues and isolated synovial fibroblast-like cells from rats with cell wall arthritis. It is also expressed intensely by chondrocytes and osteoplasts. In normal tissue it is expressed only at low levels. Transin has a strong homology to stromelysin, a metalloproteinase that is thought to play an important role in rheumatoid arthritis.

ii. MYC, a nuclear protooncogene and CIS, a protooncogene coding for the B-chain of platelet derived growth factor (PDGF) are also expressed in diseased synovial tissues in increased amounts.

iii. It had previously been shown that the synovial fibroblast-like cells from rats with arthritis had tumor-like properties. It has recently been shown that these tumor-like properties are stimulated by PDGF and fibroblast growth factor and inhibited by transforming growth factor - β. Epidermal growth factor augments the effects of PDGF. On the other hand interleukin 1, tumor necrosis factor and interferon γ, factors thought to be important in the inflammatory response, were found to have minimal or no effects on synovial fibroblast-like cell growth.

iv. When the presence of cytokines was assessed in the disease process, it was found that TGF-β and PDGF are produced locally by synovium from rats with cell wall arthritis. Northern blot analysis of RNA from diseased synovium was positive when probed for TGF-β and PDGF A and B chains. Similarly, the supernatants of explant cultures of diseased synovium contain PDGF and TGF-β. Finally, TGF-β was found to be localized diffusely throughtout the diseased synovium by immunohistochemical techniques. Perhaps the most striking new finding relates to the fact that arthritis-susceptible LEW rats but not histocompatible arthritis-resistant F344/N rats fail to significantly elevate serum corticosterone or ACTH when challenged with streptococcal cell walls, interleukin-1 and serotonin agonists. These data support the concept that the hypothalamic-pituitary response is defective in arthritis-susceptible LEW rats. Corticosteroids are potent anti-inflammatory

agents that strongly suppress Ia expression. Susceptibility to arthritis was previously shown to be highly correlated with the enhanced expression of class II MHC (Ia)

b Human Disease (66).

Attempts are being made to understand the pathology of the inflammation in tissues obtained by biopsy from the joints of patients with rheumatoid arthritis. These studies involve in situ characterization of the cells as well as in vitro culture of the cells isolated from such tissues. Also the tissues are being examined for etiologic agents, particularly bacterial cell wall peptidoglycans. Finally, collaborative studies are in progress to detect mycoplasma and viruses in the tissues or in joint fluid. Many of the studies parallel precisely the types of investigations carried out on the animal models.

The major new findings are as follows:

i. Transin, a gene expressed at high levels in invasive tumors is expressed at high levels in rheumatoid synovium and at levels several fold lower in noninvasive arthritides such as osteoarthritis.

ii. MYC, a protooncogene associated with DNA replication and SIS, a protooncogene encoding the B chain of platelet-derived growth factor are expressed at higher levels in rheumatoid synovial tissues than osteoarthritic synovial tissues.

iii. The anchorage-independent and anchorage-dependent growth of synovial fibroblasts is strongly stimulated by platelet-derived growth factor and fibroblast growth factor and is inhibited by transforming growth factor β. Epidermal growth factor augments PDGF stimulated growth. Interleukin 1 tumor necrosis and interferon γ have insignificant effects on growth but interleukin 1 and tumor necrosis factor are potent stimulators of prostaglandin E2 production in the fibroblast-like synovial cells. In the fluid from rheumatoid joints PDGF, EGF and transforming growth factor β are present in significant amounts. PDGF appears to be the major synovial fibroblast-like cell mitogen in these fluids. By a variety of criteria, it has been shown that these factors are produced locally.

iv. It appears that the CD5+ B cell subset is markedly expanded in patients with rheumatoid arthritis and that this subset spontaneously produces two types of rheumatoid factor. One of the factors is polyreactive and has a low affinity, whereas the other is more monospecific and has a higher affinity.

The significance of these observations is not yet apparent.

v. As far as the attempts to identify etiologic agents, the studies in which cultured rheumatoid synovial explants are examined for mycoplasma are in progress. The tissues of one patient with rheumatoid arthritis has grown mycoplasma pneumonii and attempts to repeat this isolation are in progress. Cultures of more than ten other cultures have been negative. Attempts to look for viral nucleic acids using the polymerase chain reaction have begun in collaboration with colleagues in the National Institute of Dental Research.

2. Therapeutic trials

a. Cyclosporin(72)

The efficacy and toxicity of daily oral cyclosporin A (CSA) in patients with active rheumatoid arthritis is being evaluated in a 6 month double-blind study of low dose (1mg/kg/day) vs. high (10 mg/kg/day) CSA. During the past year analysis of pharmacokinetic studies and immunologic effects of CSA and 12 months of clinical trials have been completed. Analysis of the clinical data from 31 patients has shown that a 40% or greater improvement in disease activity following 6 months of CSA occurred in 10 out of 15 high dose and 4 out of 16 low dose patients. Statistically significant improvement in individual measures of disease activity were documented only in the high dose group and included the number of tender and swollen joints and physician and patient global scores. Clinical responses to CSA were most evident in patients with depressed in vitro peripheral blood lymphocyte proliferative responses to soluble recall antigens. A 40% or greater improvement in disease activity developed in 8 out of 9 anergic patients compared to 6 out of 16 nonanergic patients. Improvement in disease activity was maintained through 12 months in the high dose group. Toxicities were common, particularly nephrotoxicity in which 27 out of 31 patients developed a 20% increase in serum creatinine.

b. Methotrexate (73)

In this pilot study 35 patients with severe treatment refractory rheumatoid arthritis were treated with high dose intravenous methotrexate (500 mg/m^2) followed by leucovorin (50 mg/m^2). The major findings were that the fourth course of chemotherapy was followed by a 50% or greater improvement in joint swelling, pain score and morning stiffness in all patients. The clincal responses persisted for 6 to 14 weeks posttherapy. High dose

methotrexate leucovorin was associated with a significant reduction in DR antigen expression on peripheral leu 2, leu 3 and leu 4 lymphocytes.

B. Systemic Lupus Erythematosus

1. Pathogenesis

a. Animal Models (20)

Several strains of mice spontaneously develop autoimmune diseases resembling human systemic lupus erythematosus. Early in life they are immunologically hyperactive, become relatively resistant to tolerance induction and gradually develop antinuclear and other autoantibodies followed by clinical illness. This project involves a detailed analysis of the genetic cellular and biochemical basis for the disease. During the past year the major findings were as follows:

i. The number of cells producing antibodies reactive with a panel of antigen increases with age, both in normal and autoimmune strength; however, the increase is much faster in the autoimmune strains and begins earlier - that is, within the first 2 weeks of life. Later in life there is a skewing toward autoantibody producing cells in the autoimmune strains, suggesting that polyclonal activation is the initial process and that secondarily there is antigen-driven (and T-dependent) expansion of autoantibody producing cells.

ii. One of the genes associated with the susceptibility to autoimmune disease in these animals is the gld gene. This gene has now been closely linked to the gene for antithrombin 3 on the distal mouse chromogome 1, a region that is syntactic with human chromosome 1q. Such studies will help to define the nature of the genes that lead to this enhanced susceptibility in mice and potentially of the products that they encode. It will then be of particular interest to study this gene or genes in similar chromosomal sites in human patients.

iii. NZB,BXSB, MRL/+/+, MRL/1pr/1pr, and (NZB X NZW) F1 mice all have full-length 8.4 kilobase MCF retroviral transcripts in thymus, spleen and (to a lesser extent) in other organs. Such transcripts have not previously been reported and are uncommon in nonautoimmune strains. They are present from the first day of life in NZB mice suggesting that they do not result from the disease as a secondary phenomenon. The same conclusion comes from finding such transcripts in substantial amounts in NZB.xid and BXSB.xid mice which have much reduced autoimmune

disease. Probes have been made which will are able to distinguish subgroups of MCF viruses on the basis of enb and LTR differences.

iv. In situ hybridization studies of splenic B cells show that MRL/lpr/lpr mice use 5'IGV_h and 3' IGV_h genes in proportion to their new representation in the genome. This suggests that the hyperactivity of the B cells may be general rather than specifically antigen-driven.

b. Human Disease (23)

The purpose of these studies is to understand the pathogenesis of human SLE so that prophylactic and therapeutic interventions can be maximized. These studies include analysis of genetic, immunological and environmental factors which might contribute to disease. During the past year the following new major findings have been made.

i. Patients with SLE have many abnormalities of thyroid function tests. Some of these result from systemic disease. Others are associated with thyroid stimulating and antibodies that inhibit thyrotropin binding.

ii. Patients with SLE have reduced suppressor-inducer function and reduced numbers of suppressor-inducer cells as measured by the phenotype CD4+, 2H4+. Patients with active renal disease have the greatest reduction, especially when renal disease also has been present. During the AMLR, 2H4 density usually increases in association with suppressor-inducer function. In SLE, there is a decrease (rather than increase) in 2H4 expression.

iii. Patients with SLE and JRA produce antibodies to the hnRNP core protein, A1. There appears to be no temporal association with anti-DNA antibodies produced in the same patients.

2. Therapeutic Studies

a. Animal Studies (22)

Various inbred strains of mice develop autoimmune disease resembling various symptom complexes observed in humans. Attempts to treat the autoimmune mice may suggest useful therapeutic interventions for humans. The major new findings during the past year were:

i. That TNF α, if given from early life to certain autoimmune mice has beneficial effects on

autoimmunity and survival; however, when given after the first manifestations of clinical disease are evident, there is no prolongation of survival.

ii. Methylprednisolone was able to reduce lymphadenopathy in MRL/lpr/lpr mice but did not prolong survival. There was a transient reduction in anti-DNA which returned to the levels of controls within one month.

iii. The mel-14 antibody deflected CD4- CD8- T cells of MRL/lpr/lpr mice from the lymph nodes leading to a marked reduction in lymphadenopathy. This effect is attributed to interfernce with the homing of lymphocytes to the nodes because the receptor needed to interact with the high endothelial venules was blocked.

b. Human Studies (40)

Patients with the diffuse forms of Systemic Lupus Erythematosus nephritis previously entered into therapeutic trials continued to be evaluated. New patients have been randomized to receive one of three treatments:

i. IV cyclophosphamide monthly,

ii. IV methylprednisolone monthly,

iii. Both (i.) plus (ii).

The initial treatment is for one year (twice as long as in the previous study) and retreatment is with the original drug regimen. 38 patients have been entered to date. It is too soon for data evaluation.

C. Polymyositis

1. Etiology/Pathogenesis

a) Animal studies (75)

An attempt is being made to understand how a short-lived virus can initiate a self-sustaining apprently autoimmune illness. Virus is tracked by direct hybridization of tissue extracts and of tissue sections (in situ hybridization) using radioactive RNA probes. In addition, Plotz and his colleagues have developed a polymerase chain reaction for detecting single-stranded viral RNA. The interaction between viral (EMC or Mengo) RNA and the autoantigens of human myositis (aminoacyltRNA synthetases) is being studied by in vivo charging assays. The principal findings during the past year were:

i. In mice infected with EMC-221A, viral sequences persist well after virus can no longer be recovered by viral culture.

ii. Inflammation and virus are often but not always coextensive early in infection.

iii. Late after infection, inflammation persists in heart and brain in the apparent absence of virus. When virus is found late, it may be in isolated single cells.

iv. No autoantibodies develop in infected animals. This virus and several related viruses, however, do not interact with the aminoacyl tRNA synthetases.

The significance of these studies stems from the likelihood that understanding the transition from acute viral infection to chronic apparently self-sustaining inflammation will shed light on how a virus could initiate a human autoimmune disease.

b. Studies in Humans (74)

By studying a variety of parameters of disease, Plotz and his colleagues hope to uncover subsets of patients who may have a common etiology or pathogenesis. In addition, they are trying to understand the relationship between the presence of a particular autoantibody and the disease in which it is found. Finally, they seek direct evidence for a viral cause of human myositis by probing tissues for viral nucleic acid by in situ hybridization. During the past year the following new findings have been made.

i. Studies on the autoantibody to histidyl-tRNA synthetase (anti-Jol) have shown that a) the subclass

distribution of the antibodies is typical for an antigen-driven, T cell-dependent immune response b) that the level of antibody fluctuates with disease activity, c) that the antibodies may appear before clinical disease is evident, d) that patients who have the antibodies have a clinical syndrome characterized by a severe onset frequently accompanied by lung and joint disease, often occurring in the first half of the year - that is, January to June. By contrast patients with another myositis-related antibody a) have disease limited to muscle, b) usually have an explosive onset in the last quarter of the year.

ii. They have established that the autoantibodies (anti-Jo1) characteristic of a subset of patients with polymyositis have the characteristics of an antigen-driven response. This observation is in accord with observations on several other autoantibodies in human disease. In contrast to some autoantibodies, however, the levels do fluctuate with disease activity and they have fortuitously been able to document their appearance several months before the onset of clinical disease. In this case IgM antibodies were the first to appear. Application of in situ hybridization for viruses to human tissue has been hampered by the difficulty in acquiring human specimens known to be positive. A number of purported positives are currently under study. Probes for the three most likely candidate viruses in human myositis patients - coxsackie, adeno and mumps viruses - have been obtained and are ready or being made ready for probing human biopsy specimens.

The significance of this work is that recognition of subsets may clarify the etiology and pathogenesis of this disease family and may shed light on a number of closely related rheumatologic diseases. Establishing that an autoantibody response is antigen-driven and related to disease activity enforces the view that specific etiologic agents should be sought.

2. Therapeutic Trials (76)

Severe idiopathic inflammatory myopathies (polymyositis, dermatomyositis and related diseases) that respond poorly to corticosteroids are difficult to treat. Three therapeutic trials are being undertaken for the treatment of such patients. They are: an attempt to see whether plasmapheresis or lymphopheresis improves the course of severe polymyositis or dermatomyositis. To determine whether cyclophosphamide given intravenously improves the course of patients who fail to respond to apheresis. To determine whether methotrexate given intravenously or in combination with azathioprine improves the course of patients unresponsive to apheresis and/or

conventional therapy. So far, the trial of intravenous cyclophosphamide was terminated because only one patient improved substantially and because there was considerable toxicity. These findings will be published. Patients are continuing to be entered into the apheresis and methotrexate trials at an acceptable rate. So far, there are no data to report.

D. Bone Metabolism and Fracture Healing (78)

a. Animal models

The initial investigations of the newly founded orthopedic unit under Dr. Mark Bolander have focused on the structure and function of bone and cartilage extracellular matrix proteins and the regulation of gene expression in fracture healing. The principal findings during the past year were as follows:

1. The work on the cloning and sequencing of the cDNA coding for osteonectin was completed. This protein is thought to be critical to bone structure and function. It contains several interesting features including a site for hydroxyapatite and two structures homologous to the EF-hand structures of calmodulin that are probably high affinity calcium binding regions. It is identical to a protein secreted by basement membranes previously called Sparc which is found in blood vessels, lung and cartilage. Monoclonal antibodies have been developed to osteonectin and will be used to elucidate the function of this molecule.

2. Using a variety of molecular genetic probes an analysis is being pursued in which the critical steps in fracture healing at the level of gene transcription are being identified. Northern hybridization of RNA extracted from fracture callus with cDNA probes to heat shock proteins and protooncogenes have demonstrated expression of two heat shock proteins, HSP 89 and HSP 27 during fracture repair. Two protooncogenes, N-Ras and fos, demonstrate unique patterns of expression during healing while no expression of myc, sis, erb, or other protooncogenes can be detected. Elevated transcription levels of the fos protooncogene correlate with the expression of the 27 kilodalton Heat Shock Protein on the first day after fracture. The expression of TGFβ on the third day after fracture and the initiation of endochondrial ossification on the ninth day after fracture. Immunohistochemical staining of the fracture callus with an antiphos antibody localized the protooncogene to chondrocyte subpopulations in the fracture callus suggesting that phos expression is related to certain stages of chondrocyte development. To date expression of TGF-β, and IgF-2 but not IgF-1 and both afgf and bfgf in the fracture callus have been identified. TGF-β is a major mediator in cell differentiation, proliferation and extracellular matrix production in soft tissues and while bone is the most abundant

source of TGF-β, the significance of it to bone remodeling and repair is not yet known. Analysis of TGF-β during fracture repair by Northern blots demonstrated a peak of gene expression on day 3 after fracture and high levels of expression beginning on day 10. The elevated expression on day 3 preceded the appearance of cartilage in the fracture callus while the elevation on day 10 coincided with the activation of bone-specific genes. The high levels of TGF-β were localized to the areas of enchondrial ossification. The correlation of growth factor expression with certain histological events and distinct changes in structural gene expression supports the hypothesis that these proteins have regulatory functions during fracture healing.

3. High levels of collagenase I and IV expression in the fracture callus between 4 and 7 days after fracture have been found. Indomethacin, a drug which has been shown to an adverse effect on fracture healing in animal models and to co-inhibit cyclooxygenase PGE2 and collagenase synthesis in vitro, markedly increases PGE2 levels in the fracture callus.

4. Evaluation of healing fractures in diabetic animals demonstrated a severe defect in the development of mechanical strength. This was found to correlate with a 50% decrease in collagen content and a 40% decrease in DNA content. Treatment with insulin at levels well below that required to normzlize blood glucose corrected these abnormalities. Initial studies have demonstrated a decrease in the ratio of collagen 1 mRNA/total DNA in the diabetic callus suggesting that the decrease in collagen content may be due to impaired gene expression.

5. A useful model for the study of fracture healing in osteoporosis has been developed in the estrogen-depleted female rat and is currently being studied to investigate the role of estrogen lack in producing the lesion and estrogen administration in correcting it.

b. Studies in humans

An attempt is being made to determine the resolution that would be required to make digital subtraction useful in the analysis of bone radiographs and to develop hardware and software for digital subtraction of such x-rays. Preliminary studies in cadaveric models of erosive bone diseases and in normal volunteers have demonstrated the ability to detect and quantitate localized lesions that represent bone loss of less than 3 milligrams-lesions that are below the limit of detectability on plain films. A protocol for clinical study of erosive bone lesions in patient volunteers with rheumatoid arthritis is planned.

II. Fundamental Studies (25)

The receptor for immunoglobulin E (IgE) mediates reactions of considerable medical interest; in addition, it serves as a more general model system for effector systems activated by immunoglobulins. The basic aim of the studies is to understand the early events triggered by this receptor in molecular terms. For this purpose studies are pursued for determining the structure of the receptor, exploring the early events it activates and ultimately attempting to understand the structural-functional relationships. During the past year the following new results were obtained: 1) Cloning and sequencing of the cDNA for the β subunit of the receptor was completed. 2) cloning and sequencing of the cDNA for the γ subunit was completed. 3) Expression of the receptor on cos cells transfected with the genes for the three subunits was achieved. 4) The cDNA for the human α subunit was cloned and sequenced. 5) A chimeric receptor consisting of the human α and rodent β and γ chains was expressed on cos cells. 6) The chromosomal localization of the genes coding for α,β and γ was begun. Interestingly, the genes for α and γ lie close to the genes coding for Fcγ receptors. The ability to express the gene for the human α chain in transfected cells now opens the way to developing an in vitro assay by which the binding of human IgE to human receptors can be assessed. The most obvious importance is that it is now practical to search for inhibitors of the binding. Similarly, if the expressed receptors can be shown to initiate the same biochemical cascade activated by the endogenous receptor of mast cells and basophils, the activation mechanism can be explored using genetic techniques. Ultimately, an inhibitor of this reaction can then be sought. This would also have considerable therapeutic implications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41020-21 ARB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pathogenesis of autoimmunity in mice with SLE like illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
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Seldin, Michael, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
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Klinman, Dennis, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Kurrasch, Regina, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Takashi, Tohru, Guest Researcher, Cellular Immunology Section, ARB, NIAMS

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of antibody producing cells, including autoantibody producing cells, demonstrate that murine lupus is initiated by polyclonal B cell activation which is followed by preferential (probably antigen-dependent and T cell dependent) autoantibody production.

All of the autoimmune mouse strains but not most normal strains of mice express full-length (8.4 kb) MCF retroviral transcripts in thymus, and spleen. NZB mice have such transcripts from birth suggesting that they do not result from disease. Polyclonal immune activators can induce at least one such 8.4 kb MCF retroviral transcript in NFS mice; however, additional transcript(s) are found in the unstimulated autoimmune mice.

The gld gene has been mapped to a region of distal mouse chromosome 1 which is syntatic with human chromosome 1 and with close linkage to AT3.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 41021-19 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antinuclear Antibodies in Spontaneous and Drug-induced SLE and other Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

This project has been combined with study of immune abnormalities in patients with SLE (Z01 AR 41023-14 ARB). No longer an independent project.

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminate

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41022-16 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Therapeutic Studies in Murine Lupus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, ARB, NIAMS
John D. Mountz, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
William C. Gause, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatis

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIAMS - Bldg. 10, Rm. 9N218, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cyclophosphamide is effective therapy for murine lupus.

MRL-lpr/lpr mice treated with corticosteroids from 3 months of age did not achieve significantly prolonged survival.

Others have reported that tumor necrosis factor alpha (TNF) deficiency is a fundamental defect in murine lupus and that replacement therapy is very effective in preventing disease. We have treated BXSB males, MRL-lpr/lpr mice of both sexes and (NZB x NZW)F_1 mice with TNF after the onset of disease manifestations. Such therapy was of modest benefit. Lymphadenopathy was reduced in MRL-lpr/lpr mice but survival was not prolonged in any of the strains. Increased survival was observed when younger mice were given prophylactic therapy.

In vivo administration of mel-14, an antibody to a molecule critical to lymphocyte lymph node homing, led to a marked decrease in lymphadenopathy in MRL-lpr/lpr mice.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41023-14 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Immune Abnormalities in Patients with SLE

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, ARB, NIAMS
LeeAnn Tracy, Guest Researcher, Cellular Immunology Section, ARB, NIAMS
Dennis M. Klinman, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS

COOPERATING UNITS *(if any)*

C. Morimoto, Harvard Medical School — Foreign: None

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIAMS - Bldg. 10, Rm. 9N218, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with SLE have increased numbers of B cells producing antibodies, including autoantibodies. T cells which regulate antibody production by B cells include helper cells, suppressor cells, and inducers of each. Patients with SLE have a decrease in numbers and percentages of suppressor inducer cells both functionally and as measured by cells with the phenotype CD 4+, 2H4+. This abnormality is especially common in patients with active disease and in those with renal disease (especially with disease activity).

Patients with SLE were found to produce antibodies reactive with recombinant hnRNP core protein A1. Such antibodies also were made by some patients with juvenile RA. Since this protein binds nucleic acids, it could induce anti-DNA (or vice versa) by internal image idiotypic mechanisms. We looked for, but could not find, evidence for an idiotypic induction of such antibodies: anti-DNA and anti-hnRNP core protein A1 antibodies bore no special temporal relationship with each other. Immunization of mice with recombinant hnRNP A1 protein led to large amounts of anti-hnRNP core protein suggesting that direct immunization might be the mechanism of induction in people.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41025-17 ARB

PERIOD COVERED

October 1, 2987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the cell surface receptor for IgE

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. H. Metzger, Chief, Sec. Chem Immunol. ARB/NIAMS
J.P. Kinet, Visiting Scientist ARB/NIAMS
S.C. Dreskin, Sen. Expert ARB/NIAMS
U. Blank, Guest Researcher ARB/NIAMS
L. Miller, NRC Fellow ARB/NIAMS
H. Kuster Visiting Fellow ARB/NIAMS
C. Ra, Visiting Associate ARB/NIAMS
K. White, Visiting Fellow ARB/NIAMS
B. Grasberger Biol. ARB/NIAMS
J. Kochan Hoffmann LaRoche
C. Huppi NCI

COOPERATING UNITS (if any)

Hoffmann La Roche
NCI

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Chemical Immunology

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9	9	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The receptor for immunoglobulin E (IgE) mediates reactions of considerable medical interest; in addition it serves as a more general model system for effector systems activated by immunoglobulins. Our basic aim is to understand the early events triggered by this receptor in molecular terms. For this purpose we are determining the structure of the receptor, exploring the early events it activates and ultimately will attempt to understand the structural-functional relationships. During the past year the following new results were obtained: 1. Cloning and sequencing of the cDNA for the beta subunit was completed, 2. Cloning and sequencing of the cDNA for the gamma subunit was completed, 3) Expression of the receptor on COS cells transfected with the genes for the three subunits was achieved, 4) The cDNA for the human alpha subunit was cloned and sequenced, 5) A chimeric receptor consisting of the human alpha and rodent beta and gamma chains was expressed on COS cells. 6) The chromosomal localization of the genes coding for α, β and γ has begun. Interestingly, the genes for α and γ lie close to the genes coding for Fcγ receptors. 7) Continued progress in characterizing cytoplasts which show receptor-mediated hydrolysis of phosphoinositides was achieved. The new results open a new era in the investigation of the receptor and should permit a detailed analysis of its structure-function relationships.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41040-16 ARB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of various cytotoxic drug progrms in diffuse lupus neprhitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, ARB, NIAMS
Paul H. Plotz, Chief, Connective Tissue Diseases, ARB, NIAMS
John M. Klippel, Clinical Director, ARB, NIAMS
Ronald L. Wilder, Senior Investigator, ARB, NIAMS

COOPERATING UNITS *(if any)*
James E. Balow, Senior Investigator, NIDDK
Howard A. Austin, Attending Nephrologist, Clinical Center   Foreign: NONE

LAB/BRANCH
Arthritis and Rheumatism

SECTION
Cellular Immunology

INSTITUTE AND LOCATION
NIAMS - Bldg. 10, Rm. 9N218, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects   ☒ (b) Human tissues   ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with the diffuse forms of systemic lupus erythematosus nephritis previously entered into therapeutic trials continue to be evaluated. New patients are randomized to receive one of 3 treatments: (i) IV cyclophosphamide monthly, (ii) IV methyl-prednisolone monthly, (iii) both (i) + (ii). The initial treatment is for 1 year (twice as long as the immediately previous study) and retreatment is with the original drug regimen. Thirty-eight patients have been entered to date. It is too soon for data evaluation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41048 09 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bacterial Cell Wall-Induced Arthritis and Hepatic Granulome Formation in the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	R.L. Wilder	Sr. Investigator	ARB/NIAMS
	R. Lafyatis	Med. Staff Fellow	ARB/NIAMS
	E. Remmers	Staff Fellow	ARB/NIAMS
	J. Case	Med. Staff Fellow	ARB/NIAMS
	G. Kumkumian	Biologist	ARB/NIAMS
	M. Sporn	Chief, LC/NCI	LC/NCI
	A. Roberts	Sr. Investigator	LC/NCI
	E. Sternberg	Visiting Scientist	CNB/NIMH

COOPERATING UNITS *(if any)*

Laboratory of Chemoprevention, NCI
Clinical Neurosciences Branch, NIMH

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chronic proliferative and erosive arthritis, resembling rheumatoid arthritis, can be induced in susceptible rats by systemic administration of cell wall fragments derived from a variety of bacteria. The invasive disease has many features in common with invasive tumors. In situ, the invasive lesion is composed primarily of "transformed"-appearing fibroblastlike cells, along with prominent new blood vessel development (angiogenesis). Lesser numbers of macrophages are also present. The expression of transin, MYC and SIS genes, often expressed at high levels in tumors, is also markedly enhanced in diseased synovium. In vitro, freshly explanted synovial fibroblastlike cells behave like tumor cells. They grow rapidly, do not contact inhibit, grow to high saturation densities, and form colonies when grown under anchorage-independent conditions. These properties are stimulated by platelet derived growth factor and fibroblast growth factor, and are inhibited by transforming growth factor-beta and retinoids. The local production of platelet derived growth factor and transforming growth factor-beta by diseased synovium has been directly demonstrated.

Arthritis susceptible LEW rats have been shown to have a defect in the hypothalamic-pituitary response to cell walls, that may explain their disease susceptibility. This model provides a powerful tool to investigate pathogenetic mechanisms relevant to human disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41066-06 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Synovial Tissues from Patients with RA and Related Conditions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. R.L. Wilder	Sr. Investigator	ARB/NIAMS	A. Roberts	Sr. Investigator	LC/NCI
R. Lafyatis	Med. Staff Fellow	ARB/NIAMS	M.Barile	Chief	DBP/CDVB
E. Remmers	Staff Fellow	ARB/NIAMS			
J. Case	Med. Staff Fellow	ARB/NIAMS			
G. Kumkumian	Biologist	ARB/NIAMS			
A. Notkins	Sci. Director	NIDR			
P. Casali	Sr. Investigator	LOM/NIDR			
M. Sporn	Chief	LC/NCI			

COOPERATING UNITS *(if any)*

Laboratory of Oral Biology NIDR
Laboratory of Chemoprevention NCI
Division of Bacterial Products, CDB

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The single feature most characteristic of rheumatoid arthritis is massive expansion of the synovium with local invasion and destruction of bone and cartilage. Histologically, this disease process resembles a mesenchymal neoplasm in that the sites of bone invasion are composed of transformed-appearing fibroblastlike cells and new blood vessels. This process is clearly driven by the immune and inflammatory systems, but the detailed mechanisms are not understood. We have shown that the fibroblastlike cells are, indeed, tumorlike in that they express high levels of transin, MYC and SIS in vivo, and grow in vitro like tumor cells. They grow rapidly, do not contact inhibit, grow to high saturation densities, and grow under anchorage-independent condtions. These abnormal properties are suppressed by the differentiating agent, retinoic acid, and by transforming growth factor beta (TGF-beta). The cells also lose their abnormal properties on passage in culture, i.e., the abnormal phenotype is reversible. Normal synovial cells can be induced to exhibit the abnormal phenotype by stimulation with platelet derived growth factor (PDGF). We have demonstrated PDGF and TGF-beta in rheumatoid synovial fluids and tissues.

In other studies, we have shown that the peripheral blood of rheumatoid patients have a markedly expanded population of CD5+ B cells which make both monoreactive and polyreactive rheumatoid factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41069-04 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The role of viruses in the induction of autoantibodies in humans and mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated - Incorporated into AR 41074 and 41075

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41071 03 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A controlled trial of apheresis in treatment of poly/dermatomyositis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* (Name, title, laboratory, and institute affiliation)

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated - Incorporated into AR 41076

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41072-04 ARB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cyclosporin A in Rheumatoid Arthritis (RA)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.H. Klippel, Senior Investigator, ARB/NIAMS
R.L. Wilder, Senior Investigator, ARB/NIAMS
D. Yocum, Guest Staff, ARB/NIAMS
L. Gerber, Chief, Rehab/CC
J. Minor, Pharmacy/CC
C. Yarboro, Nursing/CC
C. Berkebile, Nursing/CC
S.M. Wahl. CIS/NIDG
H.G. Preuss, GT. University

COOPERATING UNITS *(if any)*
NIDR,
Rehabilitation/CC, Pharmacy/CC, Nursing/CC   Foreign: None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIAMS/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The efficacy and toxicity of daily, oral cyclosporin A (CSA) in patients with severe, treatment-refractory rheumatoid arthritis is being evaluated in a 6 month double-blind study of low dose (1mg/kg/day) versus high dose (10mg/kg/day) CSA. Patients with a 40%, or greater, improvement in disease activity following 6 months of drug therapy are given the option to continue CSA for an additional 6 months. The effects of CSA on various measures of T lymphocyte function in peripheral blood is being investigated.

During the past year, analysis of pharmacokinetic studies and immunologic effects of CSA and the 12 months clinical trial have been completed and presented at scientific meetings. The manuscript detailing the clinical results of the trial has been completed and accepted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41073-03 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

High dose methotrexate with leucovorin rescue in rheumatoid arthritis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.H. Klippel, Senior Investigator, ARB/NIAMS
J.B. Shiroky, Former LOM/NIDR
B.A. Chabner, DCT/NCI
C.J. Allerga, DCT/NCI
G.Inghirami, KDS/NIDDK

COOPERATING UNITS (if any)

DCT/NCI, KDS/NIDDK Foreign: None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this pilot study, five patients with severe, treatment refractory rheumatoid arthritis were treated with high dose intravenous methotrexate (500 mg/m^2) followed by leucovorin (50 mg/m^2). During the past year, this project was brought to completion by publication of the results of the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41074 01 ARB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on etiology and pathogenesis of idiopathic inflammatory myopathy in humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P.H. Plotz	Sr. Investigator	ARB/NIAMS
	F.W. Miller	Sr. Staff Fellow	ARB/NIAMS
	T. Biswas	Visiting Associate	ARB/NIAMS
	R. Leff	Med. Staff Fellow	ARB/NIAMS
	D. Fraser	Med. Staff Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*
Nursing Unit Clinical Center NIH
Division of Rheumatology, Oklahoma Medical Research Foundation

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3
PROFESSIONAL: 2
OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Idiopathic inflammatory myopathy (poly/dermatomyositis) is an inflammatory disease of muscle in which characteristic autoantibodies occur. By applying a broad range of immunological, clinical, and epidemiological observations to a large (about 150) group of patients, we have developed evidence for subsets of patients which strongly suggest that the disease can be divided into more meaningful groups with different etiology and pathogenesis. So far, the groups of patients with anti-Jo-1 antibodies and with antibodies to signal recognition peptide are clinically and epidemiologically as well as serologically distinct from each other and from other patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41075 01 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Picornavirus-induced chronic inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P.H. Plotz	Sr. Investigator,	ARB/NIAMS
	F.W. Miller	Sr. Staff Fellow	ARB/NIAMS
	L. Love	NRSA	ARB/NIAMS
	R. Leff	Med. Staff Fellow	ARB/NIAMS
	D. Fraser	Med. Staff Fellow	ARB/NIAMS
	D. Smith	Sp. Volunteer	ARB/NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of a study of the pathogenesis of chronic inflammation initiated by a picornavirus, we have tracked the presence of viral genome by in situ hybridization after infection of mice with encephalomyocarditis virus, strain EMC-221A. Viral nucleic acid can be found in cells at least a week or two after disappearance of culturable virus. Inflammation disappears in some organs when virus does, but persists in others for much longer periods. We have worked out a variation of the polymerase chain eaction to detect persistent viral nucleic acid sequences with much higher sensitivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41076 01 ARB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Therapeutic trials in idiopathic inflammatory myopathies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P.H. Plotz	Sr. Investigator	ARB/NIAMS
	D. Fraser	Med. Staff Fellow	ARB/NIAMS
	R. Leff	Med. Staff Fellow	ARB/NIAMS
	F.W. Miller	Sr. Staff Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*

Blood Bank Clinical Center, NIH
Dept. of Rehabilitation Clinical Center, NIH
Unit of NM Diseases, NINCDS

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Section on Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Severe idiopathic inflammatory myopathies (polymyositis, dermatomyositis, and related diseases) that respond poorly to corticosteroids are difficult to treat. We have undertaken three therapeutic trials for the treatment of such patients: a) a controlled double-blind trial of plasmapheresis and leukapheresis; b) an open trial of monthly intravenous cyclophosphamide; c) a controlled, crossover trial of intravenous methotrexate with leucovorin rescue and a combination of methotrexate and azathioprine. The trial of apheresis continues; 30 patients have entered so far, with a goal of 40. The trial of cyclophosphamide was terminated after 11 patients had entered because of inefficacy. The trial of i.v. methotrexate and combination therapy, begun in September 1987, has had 11 patients entered so far.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41077 01 ARB

PERIOD COVERED October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Gene Expression in Normal and Impaired Fracture Healing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark E. Bolander	Senior staff fellow	ARB, NIAMS
Others:	Lance R. Macey	Volunteer	ARB, NIAMS
	Seiya Jingushi	Visiting Fellow	ARB, NIAMS
	Michael Joyce	IRTA	ARB, NIAMS
	Gabor G. Nemeth	Volunteer	ARB, NIAMS

COOPERATING UNITS *(if any)*
Laboratory of Developmental Biology and Anomalies, NIDR (Y. Yamada, G. Martin and W. Horton)
Department of Orthopaedic Surgery, Georgetown University (S.Kana, R. Stinger)
Laboratory of Chemoprevention, NCI (A. Roberts and M. Sporn)

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Orthopaedic Research Unit

INSTITUTE AND LOCATION
NIAMS, NIH Bethesda, Md.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Orthopaedic Unit has developed a research program conducting basic investigation at the cellular and molecular levels into the pathophysiology of bone formation, remodeling, and repair. Initial investigations have focused on the structure and function of bone and cartilage extracellular matrix proteins and the regulation of gene expression in fracture healing. Additional efforts are directed towards the study of wound healing in other musculoskeletal tissues.

In FY88 we published the nucleotide and amino acid sequence of osteonectin, a major protein component of the bone extracellular matrix, and suggested a model for osteonectin structure. Surprisingly, we found identity between osteonectin and a protein secreted by basement membranes, called SPARC, that is found in blood vessels, lung, and cartilage. We have developed monoclonal antibodies to osteonectin, and are using them to elucidate the function of this molecule.

Using recombinant DNA technology we have found that a temporal sequence of gene activity is observable during repair of bone injury, and that this sequence is altered in models of abnormal healing, including diabetes and after adriamycin administration, and in fractures that heal by intermembranous bone formation. We have demonstrated that in impaired fracture healing this alteration in gene expression correlates with abnormalities in protein content and mechanical strength. We developed methods for organ culture of fracture callus, and have demonstrated the effects of growth factors on the expression of structural protein genes during normal fracture repair.

We have identified expression of TGF-B, IGF-2 but not IGF-1, and both aFGF and bFGF in the fracture callus. High levels of TGF-ß were localized to areas of endochondral ossification, where osteoblasts, osteoid, and hypertrophic chondrocytes immediately adjacent to regions of calcified cartilage showed strong staining with anti-TGF-ß antibody. The correlation of growth factor expression with certain histological events and distinct changes in structural

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41078 01 ARB

PERIOD COVERED October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The application of digital subtraction techniques to bone radiography

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mark E. Bolander Senior staff fellow ARB, NIAMS

COOPERATING UNITS *(if any)*

Diagnostic Systems Branch, NIDR (R. Weber, U. Ruttimann, and H. Himel)
Department of Orthopaedic Surgery, Georgetown University (S.Kana, R. Stinger)

LAB/BRANCH Arthritis and Rheumatism Branch

SECTION Orthopaedic Research Unit

INSTITUTE AND LOCATION NIAMS, NIH Bethesda, Md.

TOTAL MAN-YEARS: .5	PROFESSIONAL: .5	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Digital subtraction radiography has proved to be a powerful technique in several areas, ie. for analyzing images after injection of contrast material in angiography. Digital subtraction has not been applied to the analysis of bone radiographs because it has not been possible to maintaining proper registration of the object in serial radiographs. We have undertaken studies to determine the resolution that would be required to make digital subtraction useful in the analysis of bone radiographs, and have collaborated in studies developing hardware and software for digital subtraction of bone radiographs. Preliminary studies in cadaveric models of erosive bone diseases and in normal volunteers have demonstrated the ability to detect and quantitate localized lesions that represent bone loss of less than 3 mg, lesions that are below the limit of detectability on plain films. A protocol for clinical study of erosive bone lesions in patient volunteers with rheumatoid arthritis has been approved, and patients will soon be registered for this project.

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